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For: IN VITRO REPAIR OF BONE AND/OR CARTILAGE DEFECTS

Assistant Commissioner for Patents
Washington, D.C. 20231

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A METHOD FOR IN VIVO REPAIR OF CARTILAGE DEFECTS OR BONE AND CARTILAGE DEFECTS IN NON-HUMAN MAMMAL JOINTS

Field of invention

The present invention relates to methods and materials for in vivo repair of cartilage as well as bone and cartilage defects in joints in non-human mammals, specifically horses.

Background of the invention

Among different breeds of horses in U.S. and in Europe there are around five (5) million registered "expensive" Thoroughbred Racehorses used in horse races, whereof an estimated 60% is directly or indirectly owned by U.S. horse-owners. Of all breeds Thoroughbreds is the most frequent sufferer of degenerative joint disease, mostly in the form of osteochondritis dissecans (OCD). The most often joint affected is the so-called stifle joint (femoropatellar joint, hind leg); see figure 1. The most common age for horses and especially racehorses to develop OCD is between 1 and 6 years old.
The earlier, the training of thoroughbreds is started (1 year old or less) the more frequent the disorder will appear.

This condition leaves a thickened retained cartilage of the affected focal area, which then loosens and becomes a fragment, and an osteochondritis dissecans (OCD) defect appears in the joints as seen in figure 2 below. However, the cause for the disorder is sometimes more complex. OCD has appeared to develop lesions where the condition prior to OCD has not been a thickening of the cartilage, but seems to have developed after an enchondral ossification has been complete, and where the cartilage surface has appeared normal in thickness. The important situation is that these lesions do occur in the joint quite frequently. Actually, among thoroughbreds and warmblood racehorses the condition is observed in up to 90% of the horses, especially if they begin strenuous training early in age, such as for instance from ages of 1 to 3 year old.

OCD is seen in approximately 60% of 1 year age, or less, especially if race training of the horses start when the horses are 1 year or less of age. These horses are subjected to 4 to 6 orthopedic surgical interventions, and will after these interventions not rehabilitate sufficiently, already after 2 to 3 interventions. However, it is not only the younger horses that develop OCD. It is also seen in up to 6 to 8 year old horses. In athletic horses (other than racehorses) the frequency of OCD is nearly 40%.

When horses show up with OCD joint symptoms in younger age, they tend to have more severe lesions. Clinically, the horses present with joint swelling, due to synovial effusion and varying degrees of lameness. Severe cases can be very lame and be misinterpreted as wobblers because of the difficulty they have flexing the stifles in getting up and down. In older horses, an increase in the level of exercise may be part of the history. Horses will often have a bunny-hop gait behind which sometimes is confused with neurological symptoms.

Careful examination of the joint may identify free bodies or irregularities of the cartilage surface. The most common location is on the lateral trochlear ridge of the femur and shows up as a flattened, irregular or concave cartilage surface. Generally the extent of damage to the joint identified at surgery is more extensive than findings revealed by x-rays.

In general surgery including arthroscopic intervention is used in most of the cases with clinical symptoms. The current treatment is debridement down to the tidemark of the cartilage or down to clinically healthy tissue. Loose fragments are removed. The animals are usually stall rested for two weeks after surgery at which time walking is started. Restricted exercise is continued for another two to three months, whereupon training is started or the horse is turned out, depending on the extent of the damage. Around 64% of the horses treated return to their previous use (racing, etc.). Approximately 35 % of the horses cannot return to their previous use within racing or with less possibilities of obtaining previous levels of racing.

In the fetlock joint is commonly found fragmentation and irregularities of the cartilage on the distodorsal front part of the sagittal ridge and of the condyles at the bottom of the cannon bones; an intra-articular part of the fetlock. This condition is seen in all breeds of horses, but it is more common in Thoroughbreds and Arabians.

A second condition that can be observed, described as OCD is fragmentation at the back of the fetlock off the proximal or plantar aspect of the first phalanx or long pastern bone. A third, often trauma related condition of racehorses is cartilage damage to the cannon bone condyles, which actually is not a true OCD.

OCD in shoulder joints often affects large areas of the joint surface and secondary osteoarthritis is common. It is less common than the other conditions described, but seems to affect Thoroughbreds and Quarter Horses with a similar incidence.

Conservative or medicinal treatment has only a limited effect and is mainly targeted at keeping the horse free from pains. The drugs are typically non-steroidal anti-inflammatory drugs (NSAID). Surgery of the shoulder is difficult due to the depth of the joint below the muscles in the area. However, surgery can be performed in younger horses with lesser muscle mass.

The effect on equine articular cartilage repair in joints medicated with polysulfated glycosaminoglycan (PSGAG) or sodium hyaluronate (SH) has been tested at Ohio State University, College of Veterinary Medicine, Columbus, Ohio. Results of this study indicated that no beneficial effect on clinical parameters, the gross appearance, or the microscopic appearance of defects when compared to saline controls. Some evidence from this study suggests that intra-articular PSGAG may have a detrimental effect on the healing of articular cartilage.

Occurrence of subchondral cystic lesions, also called bone cysts or osseous cyst-like lesions is commonly recognised abnormalities of bones and joints that may or may not cause lameness. The most troublesome cysts are articular cysts. Controversy exists as to whether these lesions are a manifestation osteochondritis secondary to a joint trauma, or a combination of stress and trauma.

A method of regenerative-treatment of cartilage and bone in horses with OCD and other cartilage and bone destructive conditions, such as osteoarthritis, would be an advantage. If regenerative cartilage treatment is done in young horses, during the ages where OCD is predominantly observed (i.e., 1 to 4 year old horses). This would reduce the numbers of orthopedic surgical re-interventions needed, as well as get horses back to their previous performance as racehorses or athletic horses, as well as companion horses. In racehorses it would mean that the horses could be able to return to their previous top-tuned race condition and not be inhibited from running at the high speed that they could prior to the development of OCD.

Summary of the invention

The present invention refers to a method and materials for regenerative-treatment of cartilage and bone in non-human mammals, specifically horses.
Conditions that may be treated according to the present invention are conditions, such as OCD and other cartilage and bone destructive conditions, e.g. osteoarthritis.

The present invention relates to a method and materials, which are adapted to achieve an optimal hyalin articular cartilage as a result of cartilage repair. According to the invention, the environment with which implanted chondrocytes are in contact is ape-

cially designed to obtain a hyalin cartilage structure by securing that the chondrocytes integrin receptors are exposed to a certain motif. This motif will induce signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products, which form hyalin cartilage. Hyalin cartilage is the cartilage naturally occurring in any movable joints in mammals as a thin layer that covers the ends of bones and is a hard, smooth, tough and elastic material. Opposing hyalin cartilage surfaces co-operating in a joint permit, in the presence of synovial fluid, a practically frictionless motion. Even thin layers of hyalin cartilage are capable of absorbing load forces five times the body weight. Hyalin cartilage in a joint is produced and maintained by a relatively small proportion, of the order of 1-5% by weight of the cartilage, of chondrocytes as the only living element. The hyalin articular cartilage structure is formed from matrix products, in particular collagen type II, which are secreted by chondrocytes when induced correctly. In the present context, the correct induction derives from certain proteins capable of presenting a particular recognition motif.

According to the invention, particular measure is taken to perform the cartilage repair using techniques and material, which secure that the chondrocytes are induced correctly.

One aspect of the invention relates to a method for in vivo repair of cartilage defects in joints in non-human mammals, comprising

applying, over a cartilage-defect surface part of a joint in a non-human mammal, a membrane a first surface part of which facing the cartilage-defect surface carries a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage,

introducing, in the interstice between the first membrane surface part and the cartilage-defect surface, a suspension of chondrocytes,

and joining a rim part of the membrane to surrounding intact cartilage so as to sealingly entrap the chondrocyte suspension in the interstice, thereby allowing the condrocyte suspension to produce and secrete matrix products which form hyalin cartilage.

Another aspect of the present invention refers to a method for in vivo repair of cartilage defects joints in non-human mammals, comprising

applying over a cartilage-defect surface part of a joint in a non-human mammal, a liquid composition comprising chondrocytes; at least one substance which is capable of inducing signal transduction in chondrocytes producing and secreting matrix products which form hyaline articular cartilage; and at least one substance which is capable of gelling, thereby allowing the chondrocytes to be entrapped.

Yet another aspect of the present invention is the use of a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage for the preparation of a membrane carrying the composition on at least one surface part for use as an implantable surgical material for the repair of chondral or osteochronral defects or for implantation treatment of osteoarthrosis in non-human mammals.

Further aspects of the present invention will appear from the appended claims.

Brief description of the drawings

The present invention is further illustrated by the following figures in the appended drawings wherein:

Figure 1 shows the most frequently parts of a horse affected with OCD;

Figure 2 shows a joint in detail affected with OCD;

Figure 3 shows OCD in horses in further detail;

Figure 4 shows an embodiment of the present invention, wherein the composition comprises a gelling substance;

Figure 5 shows an embodiment of the present invention including two membranes;

Figure 6 shows one embodiment of the method or use according to the present invention;

Figure 7 shows another embodiment of the present invention applicable for the treatment of osteoarthritis; and

Figure 8 shows yet another embodiment of the present invention using a membrane where both surfaces are impregnated with the hyalin cartilage inducing substance.

Detailed description of the invention

According to the invention, cartilage cells from younger animals, such as pigs and calves are cultured in the appropriate medium. The number of cells obtained by culturing from mammals are considerably higher than the number that one can obtain by culturing human chondrocytes for autologous implantation. The cartilage

cells cultured for horses are used for autologous implantation, but it might be conceivable that allogeneic cartilage cells may be used between inbreds. The same applies for other animals.

One aspect of the present invention relates to a method and materials where implanted chondrocytes are in contact with matrix material, especially designed to obtain a hyalin articular cartilage.
Another aspect of the present invention relates to methods and materials, which are adapted to achieve an optimal hyalin articular cartilage as a result of the cartilage cell implantation. According to the invention, the environment with which implanted cartilage cells, also called chondrocytes, could be combined, could be a periosteal flap from the femoral or tibial bone. The same method used by Brittberg and Peterson on humans (Brittberg, M. et al. New Engl. J. Med., 1994,331,889).

This invention includes the aspect with implanted chondrocytes' integrin receptors, exposed to a certain biochemical or mechanical structure, which creates a motif which will induce signal transduction in animal cartilage cells resulting in these cells to produce and secret matrix products. In the case of horses the motif used shall be capable of inducing and secreting matrix products from horse chondrocytes. The same applies to other animals.

The cartilage defect, accompanied by the loosened cartilage fragment as shown in figure, is debrided and the fragment removed, so that the cartilage defect now appears as an identation in the cartilage surface, often down to the tidemark zone. The circumference of the indentation normally is surrounded by healthy cartilage, and irregular adherent fragments are removed during the debridement process.

The cartilage-defect surface part of the joint may also be a surface part where the original cartilage had been torn apart. As a preparation for the repair, the defect is debrided so that the cartilage-defect surface part now appears as a cartilage-free indentation, often down to tidemark or calcified layer, the indentation being surrounded by healthy cartilage.

The membrane applied over the cartilage defect and debrided area is normally adapted in size so that the rim part of the membrane can be sutured to the surrounding healthy cartilage. The edge of the membrane is then sealed with a "suitable" glue such as a fibrin glue, such as Tisseel (Baxter Immuno Austria, a lyophilized virus-inactivated substance that consists of fibrinogen, plasmafibronectin, factor VIII, and plasminogen, which during application is mixed with

aprotinin solution, thrombin 4, thrombin 500 and calcium chloride solution, using a dual syringe system connected to a blunt injection needle). A small part of the sealed circumference is left open, just big enough to allow a needle attached to the syringe containing chondrocytes, e.g. cultured horse chondrocytes, to introduce the cells below the membrane. The opening is sealed with the glue, such as Tisseel, when retracting the needle so that the sealing of the entire edge of the membrane is completed.

A first surface part of the membrane, normally the whole area of one of the surfaces of the membrane, carries the composition comprising at least one substance which is capable of inducing signal transduction in chrondrocytes resulting in the chrondrocytes producing and secreting such matrix products which form hyalin cartilage. The membrane may have been impregnated with the composition prior to use, or the composition may be applied directly prior to or directly after application of the membrane to the cartilage-defect surface part of the joint. The membrane is a non-immunogenic, non-toxic, biodegradable membrane. The membrane may be porous or substantially non-porous, but not with a dense structure, which would prevent chondrocytes from invading the membrane.

The membrane used may for instance be of collagen type I. However the membrane should preferably be coated with a protein or protein composition that induces phenotypic maintenance of the chondrocytes, so that no shift towards fibroblasts or osteoblasts/osteocytes is induced. Chondrocytes, bone cells and fibroblasts are all from the same mesenchymal stem cell. The coating or impregnation of the membrane or generally application of the membrane may be performed by spraying, painting or immersion. The structure of the membrane is then changed in its ability to allow chondrocytes to adhere to and invade the entire membrane. By allowing chondrocytes to invade through the membrane, a particularly smooth surface of cartilage, levelled to the surrounding cartilage can be obtained.

Alternatively, the chondrocyte suspension may be applied simultaneously with the solution containing the motif or motifs using a twin syringe.

One substance that is capable of inducing the signal transduction is preferably a peptide or protein containing a motif recognised by integrin receptor sites of chondrocyte membranes, such as the sequence Arg-Gly-Asp (RGD motif).

A few definitions and explanations concerning cartilage, integrins and RGD recognition motif are given here:

Cartilage:

The biologic and mechanical properties of cartilage depend on the design of the tissue and the interaction between the chondrocytes and the matrix that maintains the tissue. Chondrocytes form the macromolecular framework of the tissue matrix from three (3) classes of molecules: collagens, proteoglycans, and non-collagenous proteins. Type II, IX, and XI collagens form a fibrillar network that gives the tissue the tensile stiffness and strength. Collagen type VI forms part of the matrix immediately surrounding the chondrocytes and help the chondrocytes to bind and attach to the framework of the matrix. Of the collagens, mentioned above, collagen type II is the most abundant (Gay, S. et al. Arthritis Rheum. (1980) 23,937; Mayne, R., Cartilage Collagens – what is their function, and are they involved in aticular disease? Arthritis Rheum. (1989) 32,241).

The large aggregating proteoglycans (aggregans) give the tissue its stiffness to compression. Small proteoglycans, decorin, biglycan and fibromodulin, bind to other matrix macromolecules and help stabilize the matrix. The non-collagenous proteins including anchorin CII, tenascin and fibronectin, helps chondrocytes to attach to the matrix (Hardingham, T. and Bayliss, M., Semin. Arthritis Rheum. (1990) 20,12; Hardingham, T., et al., Eur. J. Clin. Chem. Clin. Biochem. (1994) 32,249).

Integrins:

Chondrocytes express a number of cell-surface molecules that mediate cell-cell or cell-matrix interactions. It is well known that cellular interactions, such as cell adhesion, migration, invasion, between cells and the extracellular matrix are mediated by the integrin family of cell surface receptors (Sonnenberg, A., Integrins and their ligands. Curr. Top. Microbiol. Immunol. (1993), 184,35; Springer, T.A., Nature (1990) 346, 425).

Cell adhesive interactions play important roles during many normal physiological processes such as wound repair. Cell adhesion is mediated by the specific interactions of cell surface receptors with extracellular glycoproteins. The best described cell adhesion receptors are in fact the integrins which comprise a family of more than twenty three (23) non-covalent, heterodimeric complexes consisting of an alpha and a beta subunit non-covalent bound together (Salter, D.M., et al., Integrin expression by human articular chondrocytes, Br. J. Rheumatol. (1992) 31,231; Woods, V.L. et al., Arthritis Rheum. (1994), 37,537).

RGD motif:

The integrins interact with extracellular matrix molecules, serum constituents and the adhesion molecules of the immunoglobulin family. The extracellular domains of many integrins recognise the RGD tripeptide (Arg-Gly-Asp) found in several extracellular macromolecules such as Vitronectin, Fibronectin (type I,II or III) , Fibrinogen, Fibrillin (type I and II), Kistrin, Echistatin, Von Willebrand Factor (vWF) and in the bone matrix proteins such as Osteopontin (OPN, a very abundant protein in the bone) and Bone Sialoprotein (BSP).

RGD-containing proteins have been shown to be components of cartilage matrix. Cell attachment assays have shown the presence of integrins mediating binding of chondrocytes to fibronectin in an RGD-dependent manner (Arner, E.C., et al., Arthritis Rheum. (1995) 38,1304).

Fibronectin:

The best characterised of the integrin ligands is fibronectin. Fibronectin has at least 2 independent cell adhesive regions: one located near the centre of the polypeptide chain in the 9. and 10. Type III modules binds to the alpha 5 beta 1 integrin. The biological function of the central cell adhesive region requires 2 critical amino acid sequences - an Arg-Gly-Asp (RGD) sequence and a Pro-His-Ser-Arg-Asn (PHSRN) sequence, which function in synergy for optimal binding to the alpha 5 beta 1 integrin. The spacing between the crucial RGD and PHSRN sequences is also important for activity, suggesting that the individual sequences alone are necessary, but not sufficient, to account for cell adhesive activity of fibronectin.

Although many integrins can bind fibronectin, the alpha 5, beta1 integrin is the major fibronectin receptor on most cells including chondrocytes. This integrin mediates cellular responses to e.g., fibronectin substrates as adhesion, migration, assembly of extracellular matrix, and signal transduction (Salter, D.M., et al., Br. J. Rheumatol.(1992) 31,231; Woods, V.L. Arthritis Rheum. (1994) 37,537).

Chondrocytes from animals such as horses and especially younger horses and other mammals such as pigs and cattle, especially young cattle, surprisingly expands with a significantly higher number of cells in culture, when compared to human chondrocytes. An integrate part of this invention is disclosing that due to the yield per 100 mg of cartilage can allow expansion of cultures up to 20 to 100 million cells within a 4 to 6 week period. It is often necessary to obtain larger number of cells in order to repair the larger size of defects in the animals described above, when compared to cartilage defects in humans.

In the method of the invention, the motif recognised by integrin receptor sites of chondrocyte membranes is preferably the sequence Arg-Gly-Asp (RGD). RGD is known to be recognised by various cells, including chondrocytes, and is known to induce such signal transduction that results in the correct matrix production that delivers building components for hyalin articular cartilage. However, to the knowledge of the inventors, it has never been suggested to utilise this phenomenon in chondrocyte implantation.

It will be understood that any other motif or sequence, which has the same or substantially induction capability as RGD could also be used in the method of the invention. The suitability of a motif or sequence for the purpose can be evaluated by incubating cultured chrondrocytes with a candidate substance followed by preparation of the cells for electron microscopy, and studying the chondrocytes at a suitable magnification; chondrocytes capapble of secreting matrix products can easily be recognised in the this way because of their particular structure including cupula present in the membranes of the chondrocytes.

Thus, suitable substances of the composition are peptides or proteins showing the RGD motif. The peptides or proteins may be naturally occurring or synthetically prepared ones and more specifically biodegradable natural or synthetic polymers. The peptide or peptides presenting the motif or motifs may also be presented on a carrier.

While a substance or substances showing the above-mentioned motif inducing the secretion of the correct building stones of hyalin cartilage constitute a compulsory constitutent or compulsory constitutents carried by the coated or impregnated membrane, it is advantageous that the at least one peptide or protein of the composition contains a further sequence recognised by receptor sites of chondrocyte membranes. The further sequence is preferably the sequence Pro-His-Ser-Arg-Asn (PHSRN).

In many cases, it is preferred that the motif and the further sequence are carried on the same peptide or protein. Examples of proteins showing both the RGD motif and the sequence PHSRN are collagen proteins such as types II, VI, IX, and XI, , proteoglycans such as aggregans, decorin, fibromodulin and biglycan, and non-collageneous proteins such as cryoprecipitate, fibronectin, vitronectin, fibronogen, fibrillin, kistrin, echistatin, von Willebrand factor, tenascin and anchorin CII.

It is especiallly preferred that the protein is collagen type II or fibronectin.

In addition to the membrane carrying the composition which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage, it may be preferable to apply such a composition on the cartilage-defect surface prior to the application of the coated membrane, to thereby increase the intensity of the exposure of the implanted chondrocytes to the motif, as well as the capability of the implanted cells to bind with the surrounding chondrocytes and cartilage.

The chondrocytes can also in another embodiment of the invention be implanted, together with another liquid such as collagen type II, or a protein such as fibronectin. The chondrocytes are in this embodiment mixed with the other liquid immediately prior to injecting the chondrocytes into the defect, or simultaneous to the injection of the chondrocytes into the defect. The mixture of the cells and the liquid substance consisting of for instance collagen type II is kept at low temperature (below 10 degrees C), slightly acidified and only slightly buffered, for instance in an acetic buffer with a low molarity. When exposed to a temperature higher than 10 degrees C at a neutral pH (around pH 7) the mixture will form a gel. This allow the user to fill the defect in the cartilage in the joint of a mammal in which its is difficult to get access to the defect in the joint without using an arthroscope. Again this would allow the user to fill the defect with the solidifying mixture of protein and chondrocytes, so that the solidification is happening in the defect as a clot filling the defect. It may sometimes be necessary to inject or spray a thin layer of cryoprecipitate, Tisseel, or resembling product to coat the lining of the defect in the bottom of the defect and on the side of the defect in order to glue the solidifying substance mixed with the cells resulting in a clot, filling out the entire defect and held in place by the coating glue (figure 4).

The substance inducing signal transduction in chondrocytes and the substance resulting in gelling may be the same or different.

In this and other embodiments of the invention, some of the chondrocytes to be implanted can be included in the composition carried by the membrane and optionally applied directly on the cartilage defect in order to obtain a lining of cells resulting in a normal cartilage surface layer and transitional layer.

It is also possible to add, to the chondrocyte suspension to be implanted, a suitable concentration of a substance providing the inducing motif and optionally the additional sequence, such as one of the substances discussed above, in particular fibronectin or preferably collagen type II, to thereby increase the exposure of the

implanted chondrocytes to the correct induction. The suitable concentration will normally be in the range of 10-30% of the total suspension, calculated on dry weight of e.g. collagen type II related to the total wet weight of the suspension.

A particular embodiment of the invention permits a combined repair of both bone and cartilage. This embodiment utilises two membranes (see figure 5).

Beneath the first membrane in the defect immediately over the denuded bone, cultured bone cells (osteoblasts) are injected or introduced, facing a surface of the membrane which will promote bone growth such as collagen type I. Between the first and the second (upper) membrane, covering the defect, a cultured chondrocyte suspension is implanted. The chondrocytes facing the upper surface of the first (double-layer) membrane and the lower surface of the second membrane (double-layer), both of which contain a composition, which will induce the correct cartilage matrix production.

Thus, this embodiment of the invention constitutes a method for in vivo repair of bone and cartilage defects in joints in animals, where the cartilage defect has approached the stage of osteoarthritis.

This embodiment comprising applying, over a bone and a cartilage defect surface part of which facing the bone surface part, consists of or carries a substance having substantially the growth-promoting effect on bone cells as collagen type I, such as collagen type I, and the opposite second surface part of which carries a composition comprising at least one substance, which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products, which form hyalin articular cartilage, introducing, in the interstice between the first membrane surface part and the bone, a suspension of bone cells,

joining a rim part of the membrane to surrounding intact cartilage and/or bone so as to sealingly entrap the bone cell suspension in the interstice,

applying, over the first membrane, a second membrane a first surface part of which facing the second surface part of the first membrane carries a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products, which form hyalin articular cartilage,

introducing, in the interstice between the two membranes, a suspension of chondrocytes,

joing a rim part of the membrane to surrounding intact cartilage so as to sealingly entrap the chondrocyte suspension in the interstice, thereby allowing the chondrocyte suspension to produce and secrete matrix products, which form hyaline cartilage,

and allowing the bone cells to cover the osteoarthritic bone and to grow into the first membrane and the chondrocyte suspension to produce and secrete matrix products, which form hyaline articular cartilage and chondrocytes to adhere to the second surface part of the first membrane.

The chondrocytes adhering to the second surface part of the first membrane and the bone cells are preferably allowed to eventually form a transition layer between healthy bone cells and cartilage implant in that the first membrane allows bone cells to grow into the structure of the membrane from below, and chondrocytes to adhere to and grow into the membrane from above. Thereby, transition is obtained between healthy bone cells and healthy chondrocytes.

When using titan constructions in joint replacement etc. it would, in accordance with the present invention, be possible to apply cultures of chondrocytes directly onto an irregular or rough titan surface to thereby fix the chondrocytes to said surface.

In any of the above-described embodiments, it is preferred that the cells implanted are autologous, but could in case of "close inbreds" be allogeneic cells. Bone and cartilage tissues are harvested and enzyme treated according to known methods.

The cell suspensions used are suitably cultures of the cells in question. The culturing of chondrocytes may be performed using known methods, as disclosed, e.g., by Brittberg et al. loc. cit. The culturing of bone cells may be performed using known methods, the culture medium being, e.g., Dulbecco MEM/HAM 12 with 10-20% foetal calf serum.

The cell cultures may be used as such as the suspensions implanted, or the cultures may be admixed with suitable media containing the patient's own serum at a concentration of, e.g., 10-20% vol/vol, thereby minimising immunogenic reactions. As an alternative or supplement to this, a motif-providing substance or composition

of the kind described above, such as collagen type II for chondrocyte suspensions and collagen type I for bone cell suspensions, may be added prior to implanting the suspension.

Examples of conditions resulting in cartilage defects, which may be treated according to the present invention are chondreal leasions or ostochondreal lesions, osteochondritis dissecans (OCD), chondromalacia and ostearthritis.

The principles of the invention are further illustrated in the appended drawings.

CLAIMS

1. A method for in vivo repair of cartilage defects joints in a non-human mammal, comprising,

applying over a cartilage-defect surface part of a joint in a non-human mammal, a membrane a first part of which facing the cartilage-defect surface carries a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes producing and secreting matrix products which form hyaline articular cartilage

introducing, in the interstice between the first membrane surface part and the cartilage-defect surface, a suspension of chondrocytes,

and joining a rim part of the membrane to surrounding intact cartilage so as to sealingly entrap the chondrocytes suspension in the interstice, thereby allowing the chondrocyte suspension to produce and secrete matrix products which form hyaline articular cartilage.

2. A method according to claim 1, wherein the membrane is a non-immongenic, non-toxic, biodegradable membrane, which is optionally of a structure which allows chondrocytes to adhere and invade the entire membrane.

3. A method according to claim 1 and 2, wherein the composition comprises of at least one peptide or protein containing a motif recognised by integrin receptor sites of chondrocyte membranes.

4. A method according to claim 3, wherein the motif is the sequence Arg-Gly-Asp (RGD)

5. A method according to any of the preceding claims, wherein the non-human mammal is a horse.

6. A method according to any of the preceding claims, wherein the at least one peptide or protein of the composition contains a further sequence recognised by receptor sites of chondrocyte membranes.

7. A method according to claim 6, wherein the further sequence is the sequence Pro-His-Ser-Arg-Asn (PHSRN).

8. A method according to claim 6 or 7, wherein the motif and the further sequence are carried on the same peptide or protein.

9. A method according to any of claims 3-8, wherein the protein is selected from collagen proteins such as types II, VI, IX, and XI, , proteoglycans such as aggregans, decorin, fibromodulin and biglycan, and non-collageneous proteins such as cryoprecipitate, fibronectin, vitronectin, fibronogen, fibrillin, kistrin, echistatin, von Willebrand factor, tenascin and anchorin CII.

10. A method according to claim 9, wherein the protein is collagen type II.

11. A method according to claim 9, wherein the protein is fibronectin.

12. A method according to any of the preceding claims, wherein a composition which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage is applied on the cartilage-defect surface prior to the application of the coated membrane.

13. A method according to claim 12, wherein the composition is a composition as characterised in any of claims 3-11.

14. A method for in vivo repair of cartilage defects joints in non-human mammals, comprising,
applying over a cartilage-defect surface part of a joint in a non-human mammal, a liquid composition comprising chondrocytes; at least one substance which is capable of inducing signal transduction in chondrocytes producing and secreting matrix products which form hyaline articular cartilage; and at least one substance which is capable of gelling, thereby allowing the chondrocytes to be entrapped.

15. A method according to claim 12-14, wherein the non-human mammal is a horse.

16. A method for in vivo repair of bone and cartilage defects in joints in a non-human mammal, comprising

applying, over a bone- and cartilage-defect surface part of a joint, a porous first membrane a first surface part of which facing the bone surface part consists of carries a substance having substantially the growth-promoting effect on bone cells as collagen type I, such as collagen type I, and the opposite second surface part of which carries a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage,

introducing, in the interstice between the first membrane surface part and the bone, a suspension of bone cells,

joining a rim part of the membrane to surrounding intact cartilage and/or bone so as to sealingly entrap the bone cell suspension in the interstice,

applying, over the first membrane, a second membrane a first surface part of which facing the second surface part of the first membrane carries a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage,

introducing, in the interstice between the two membranes, a suspension of chondrocytes

joining a rim part of the membrane to surrounding intact cartilage so as to sealingly entrap the chondrocyte suspension in the interstice, thereby allowing the condrocyte suspension to produce and secrete matrix products which form hyalin cartilage

and allowing the bone cells to cover the osteoarthritic bone and to grow into the first membrane and the chondrocyte suspension to produce and secrete matrix products which form hyalin cartilage and chondrocytes to adhere to the second surface part of the first membrane.

17. A method according to claim 16, wherein the non-human mammal is a horse

18. A method according to claim 16, wherein the chondrocytes adhering to the second surface part of the first membrane and the bone cells are allowed to eventually form a transition layer between healthy bone cells and cartilage

implant.

19. A method according to claim 16-18, wherein each composition is a composition as characterised in any of claims 3-11.

20. A method according to any of the preceding claims, wherein the chondrocytes suspension is a suspension of autologous chrondrocytes.

21. A method according to any of claims 16-20, wherein the bone cell suspension is a suspension of autologous bone cells.

22. A membrane at least a surface part of which carries a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage.

23. A membrane according to claim 22, which is a non-immunogenic, non-toxid, biodegradable membrane.

24. A membrane according to claim 22 or 23, wherein the membrane material is collagen type I.

25. A membrane according to claim 22 or 23, wherein the membrane material is a biodegradable synthetic polymer.

26. A membrane according to any of claims 22-25, for use as an implantable surgical material for the repair of chondral or osteochronral defects or implantation treatment of osteoarthrosis.

27. The use of a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage for the preparation of a membrane carrying the composition on at least one surface part for use as an implantable surgical material for the repair of chondral or osteochronral defects or for implantation treatment of osteoarthrosis in non-human mammals.

28. The use of a substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix

products which form hyalin cartilage for the preparation of a membrane carrying the composition on at least one surface part for use as an implantable surgical material for the repair of chondral or osteochronral defects or for implantation treatment of osteoarthrosis in non-human mammals.

29. The use of a substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage for the preparation of a composition for application to a membrane to be implanted as a surgical material for the repair of chondral or osteochronral defects or for implantation treatment of osteoarthrosis in non-human mammals.

30. The use according to any of claims 27-29, wherein the membrane is to be used as described in any of the claims 1-12.

31. The use of a chondrocyte culture for the preparation of a suspension for introduction into an interstice between a cartilage-defect surface part of a joint in a in non-human mammal and a membrane surface carrying a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage.

32. The use according to claim 31, wherein the chondrocyte culture is a culture of autologous chondrocytes.

33. The use according to any of claims 27-32, wherein the non-human mammal is a horse.

34. The use of a bone cell culture for the preparation of a suspension for introduction into an interstice between a bone- and cartilage-defect surface part of a joint and a surface part of a porous membrane, the surface part consisting of or carrying a substance having substantially the growth-promoting effect on bone cells as collagen type I, such as collagen type I.

35. The use according to claim 29, wherein the opposite surface part of the membrane carries a composition comprising at least one substance which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin

cartilage.

36. The use according to claim 29 or 30, wherein the bone cell culture is a culture of autologous bone cells.

ABSTRACT

The present invention relates to a method and materials for in vivo repair of cartilage or bone and cartilage defects in joints in non-human mammals, specifically horses. The invention involves a membrane carrying a composition comprising at least one substance, which is capable of inducing signal transduction in chondrocytes resulting in the chondrocytes producing and secreting matrix products which form hyalin cartilage. The membrane is applied over a cartilage-defect surface part of a joint and a suspension of chondrocytes is introduced between the membrane and the cartilage-defect surface.

Figure 1. The stifle joint (indicated on the hind leg) is the most frequently affected with OCD. Two other joints, also marked with arrows are also affected with OCD

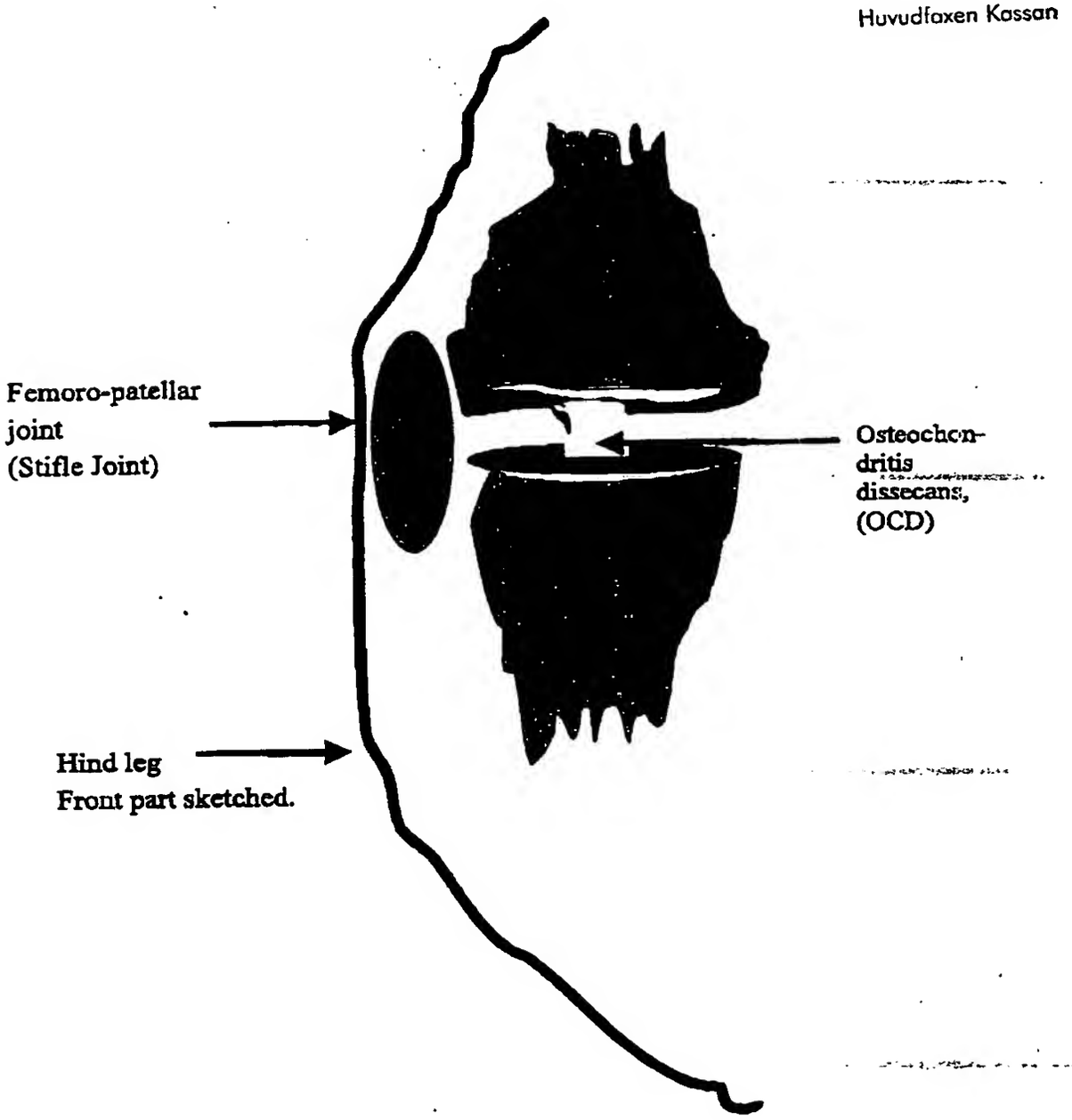


Figure 2. Horse Hind leg (Front portion: See arrow). Stifle joint with OCD, and loose fragment hanging in front part of OCD defect.

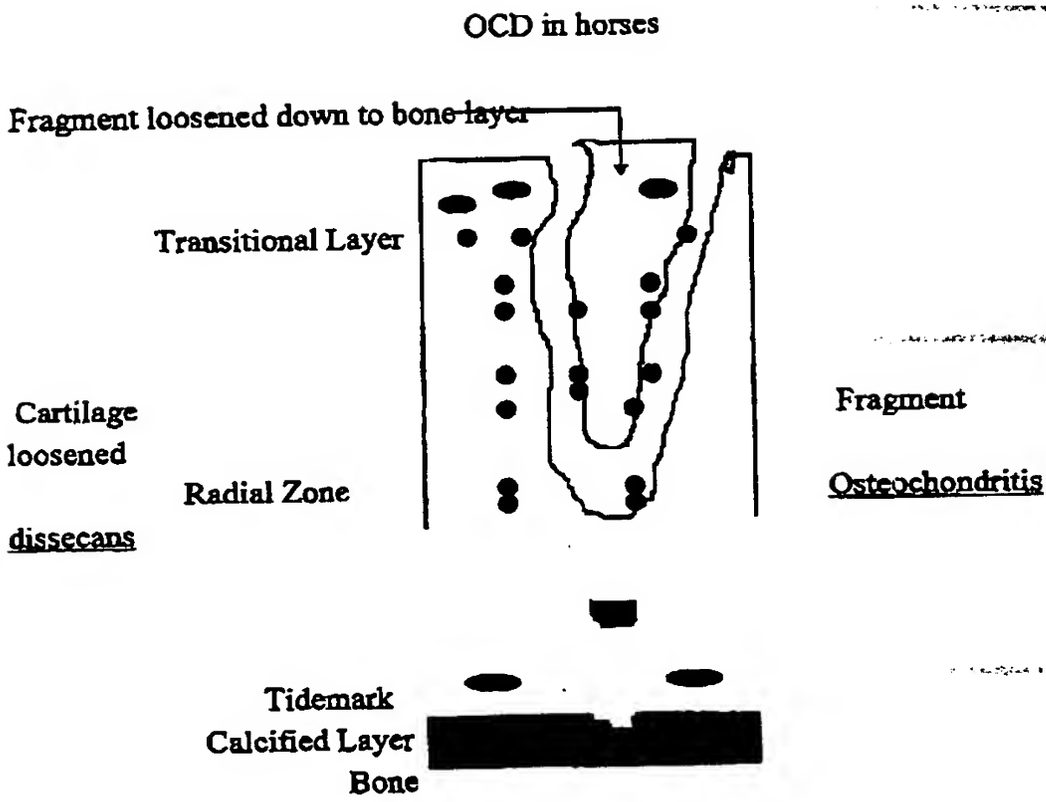


Figure 3. Osteochondritis dissecans (OCD) in the horse is characterized by a separation of a fragment of cartilage and and underlying bone from the articular surface.

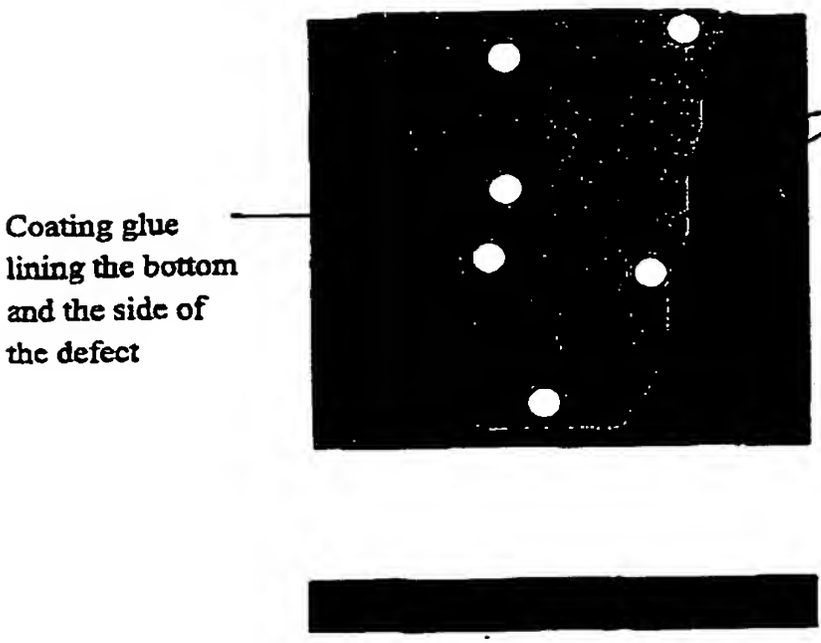


Figure 4. Arthroscopic injection of mixture of cells and protein(s) such as collagen type II into the defect after coating the bottom and the side of the defect

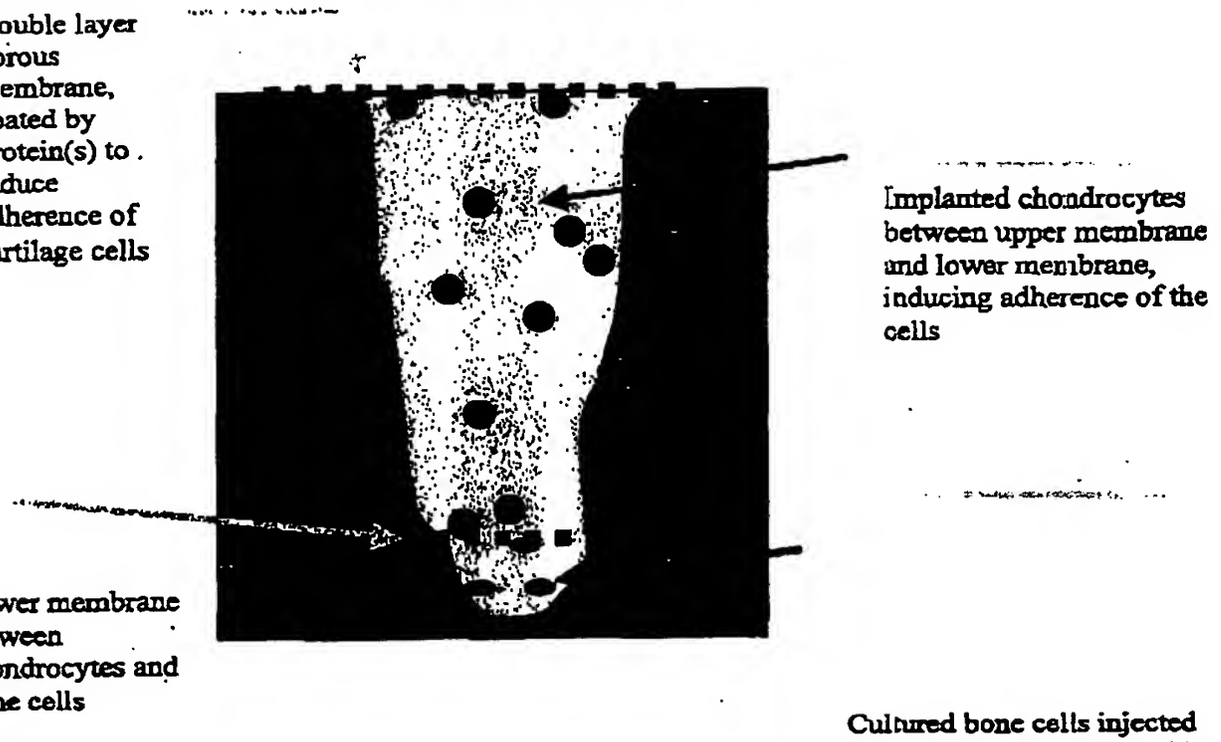


Figure 5. An upper and a lower membrane (stipled appearance) to be used in osteoarthritis development in cartilage defects in horses. Two types of cells were cultured, one being autologous chondrocytes and autologous bone cells, both induced by membrane parts (lower membrane) that promote the two types of cells to adhere and proliferate.

Figure 6
Autologous Chondrocytes – Collagen type II or Crude Cryoprecipitate or Fibronectin Induced

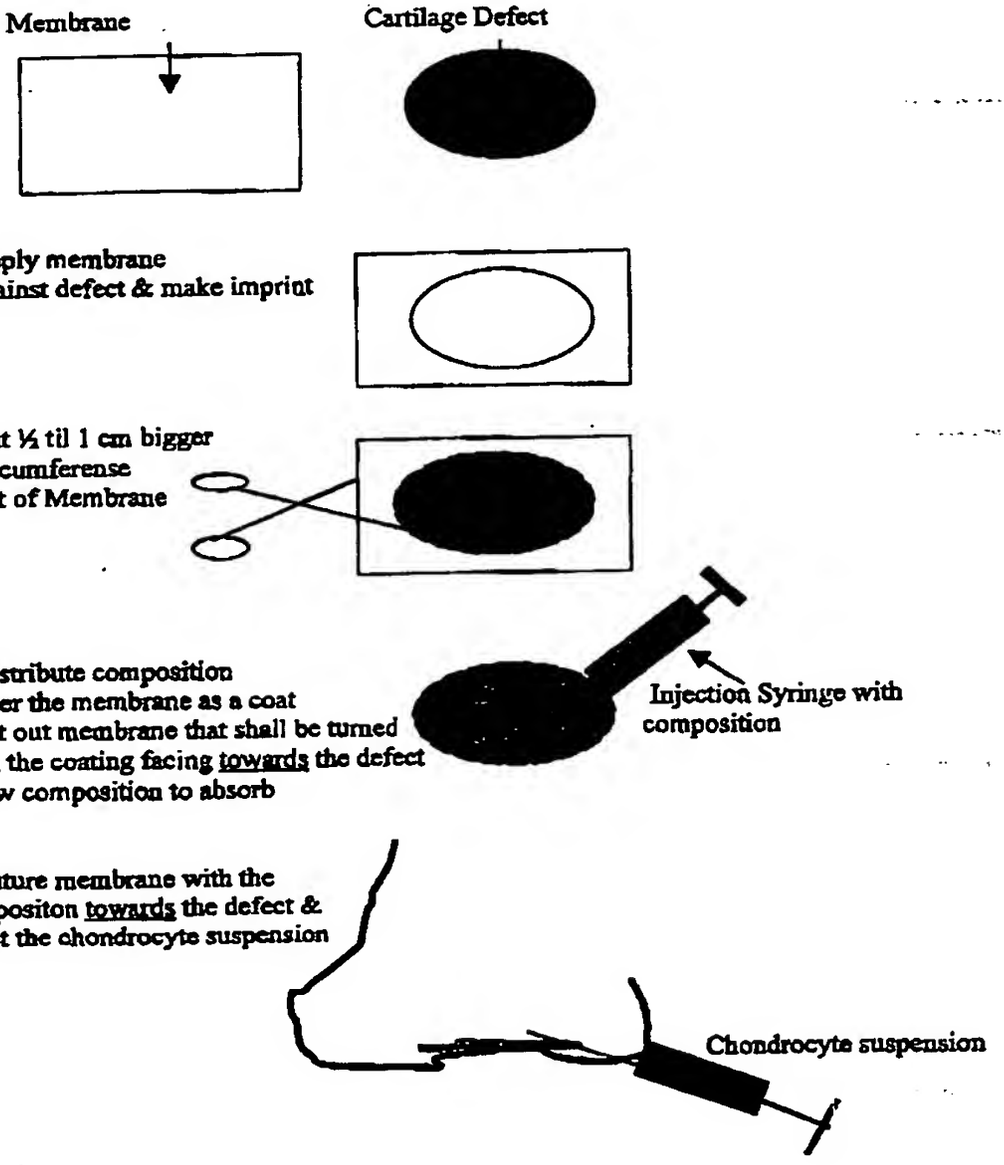
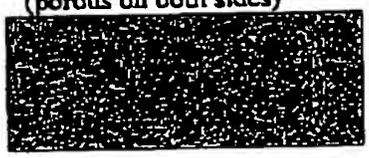


Figure 7

TREATMENT OF OSTEOARTHRITIS

1. Application of osteoblasts on the porous surface of special first membrane
2.

Special first membrane
(porous on both sides)

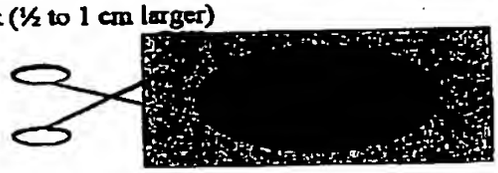


O.A. Defect

[RDG motif coated on one side (away from defect and imprint)]

2. Make imprint of defect in bottom

3. Cut out imprint (½ to 1 cm larger)



4. Place imprint in sterile Petri dish, apply Tisseel on imprint and distribute cultured immediately distribute cultured osteoblasts on Tisseel coated imprint

Distribution of Tisseel

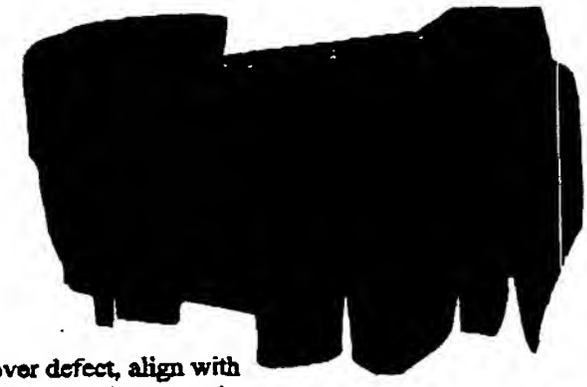
Distribution of osteoblasts

5. Leave osteoblasts on Tisseel coat for 2 minutes at room temperature

Figure 7, continued

TREATMENT OF OSTEOARTHRITIS

6. Place imprint, with osteoblast coat towards defect and RGD motif coat towards space for injected chondrocytes



7. Place second membrane over defect, align with surrounding cartilage and fasten. Inject chondrocytes into the cavity over the porous double coated membrane under which the osteoblasts are located

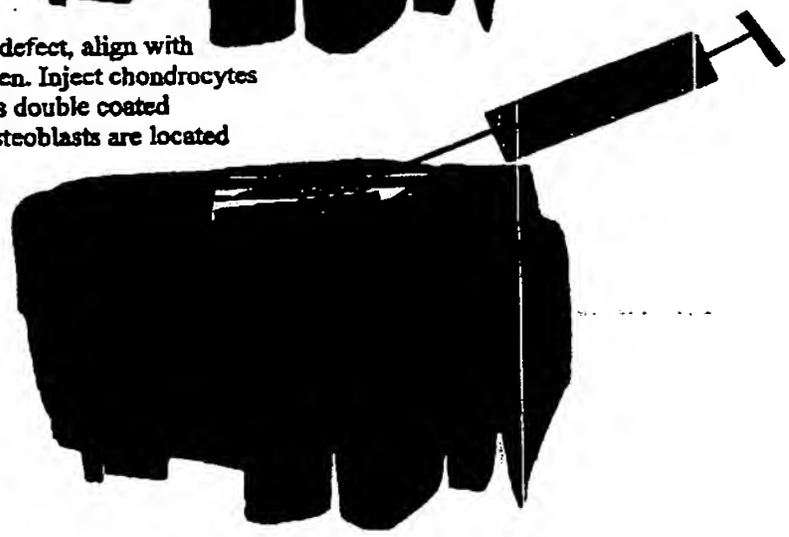
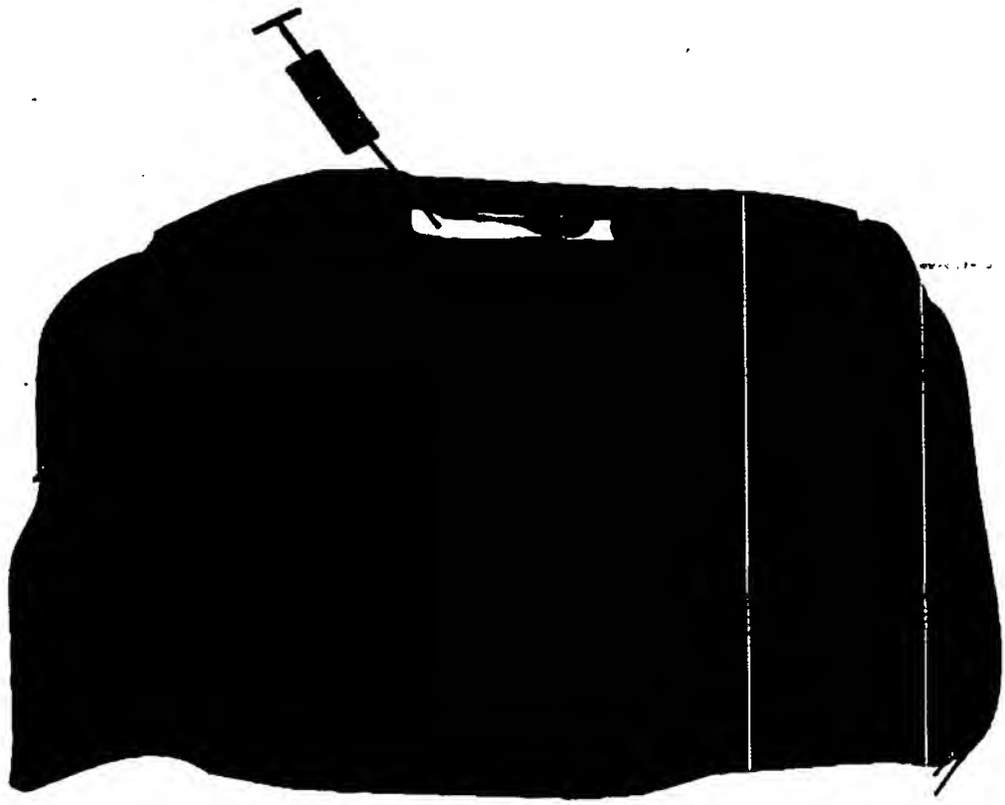


Figure 8

The use of the membrane, which is made porous on both the upper and the lower side of the membrane. The lower side of the membrane turning towards the defect (light orange) is coated with the RGD motif turning any matrix to a chondrocyte inducer for further matrix production and adhesion. The upper side of the membrane is also impregnated with RGD motif and is, as observed on the drawing, perforated or porous as is the lower part of the membrane. Chondrocytes are infused below the double porous membrane.



The perforated or porous membrane will allow chondrocytes to proliferate up to the level of the neighbouring surface. When the patient is placed in a CPM (Continuous Passive Movement) machine immediately after the implantation the implanted chondrocytes will be exposed to the same pumping mechanism that occurs over the neighboring healthy cartilage. In theory the pumping mechanism and the movement of the joint will decide the layering of cells.